DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: April 20, 2004

From: Division of Petition Review (HFS-265)
Chemistry Review Team

Subject: FAP 4A4754; Hyman, Phelps and McNamara, P.C., on behalf of zuChem, Inc., Submission of 12/11/2003. Mannitol produced by fermentation using the microorganism *Lactobacillus intermedius (fermentum)*.

ReF 2

To: Division of Petition Review (HFS-265)
Regulatory Group II
Attention :C. Johnston

Introduction

Mannitol was determined to be generally recognized as safe (GRAS) based upon evaluation of information on its use and safety during the agency's comprehensive review of direct human food ingredients (38 FR 20046, July 26, 1973). However, the GRAS status was subsequently revoked, and an interim food additive regulation (21 CFR 121.4005, now 21 CFR 180.25) was granted (39 FR 34178, Sept. 23, 1974). The regulation was subsequently amended (61 FR 7991, March 1, 1996) to allow for the use of mannitol produced by fermentation using the yeast *Zygosaccharomyces rouxii.*

In the current petition, Hyman, Phelps and McNamara, P.C., on behalf of zuChem, Inc. (zuChem), is petitioning to amend §180.25 to include mannitol produced by fermentation using the microorganism *Lactobacillus intermedius (fermentum).*[1] Mannitol produced using zuChem's method would be used in the same foods and at the same levels specified in §180.25(d).

Identity

Mannitol

Mannitol produced by fermentation is the same substance identified in §180.25(a) (i.e., 1,2,3,4,5,6-hexanehexol, $C_6H_{14}O_6$). Mannitol is identified by the Chemical Abstracts Service (CAS) number 69-65-8, has a molecular weight of 182.17, and is known by the synonyms D-mannitol, mannite, manna sugar, and cordycepic acid.

Fermentation Microorganism

[1] As discussed in Section E of the petition (p. 000009), the microorganism used in zuChem's fermentation process was formerly classified as *Lactobacillus intermedius*, but is now known as *Lactobacillus fermentum*. For the remainder of this memorandum, we shall refer to the organism as *Lactobacillus fermentum*.

The microorganism has been identified as *Lactobacillus fermentum* (Section E (p. 000009) and Appendix E, Attachment 1 (pp. 000110-113)).[1] ZuChem indicates that the microorganism has been identified in various food products including sourdough bread, fermented maize dough, cheese, and malt whiskey. ZuChem also notes that a urease preparation derived from *Lactobacillus fermentum* for use in winemaking is listed as GRAS in 21 CFR 184.1924.

The identity of the fermentation microorganism will be described by an Office of Food Additive Safety (OFAS) microbiologist. We defer to the OFAS microbiologist regarding the identity of the fermentation microorganism.

Manufacturing

ZuChem states that they have developed an efficient method for the production of mannitol using a strain of *Lactobacillus fermentum* which converts D-fructose to D-mannitol. The manufacturing process is described in Appendix A (pp. 000017-22). The primary sugar substrate used in the production of mannitol is fructose[2], but secondary sugar sources can also include glucose, maltose, mannose, raffinose and galactose.

We will rely on the OFAS microbiologist to describe the fermentation process in detail, but we will briefly describe the manufacturing process. A flow chart of the manufacturing process is provided in Appendix A (p. 000022).[3]

Specifications

Although §180.25(b) specifies that mannitol must meet the specifications of the Third Edition

[2] ZuChem states (p. 000019) that the sugar feed is typically a mixture of high fructose corn syrup (which contains fructose and glucose) and liquid fructose in a fructose:glucose ratio ranging from 3:1 to 2:1.

[3] Although not specifically discussed in the petition, a referenced article (Saha, B.C., L.K. Nakamura, "Production of mannitol and lactic acid by fermentation with *Lactobacillus intermedius* NRRL B-3693. *Biotech. Bioengineer.* 82(7): 864-871, 2003) discusses the efficiency of the fermentation process. According to the article, *Lactobacillus intermedius* converts a 2:1 mixture of fructose and glucose into mannitol, lactic acid, acetic acid and carbon dioxide at the theoretical ratios of:

2 Fructose + 1 Glucose → 2 Mannitol + Lactic Acid + Acetic Acid + Carbon Dioxide

of the Food Chemicals Codex (FCC III), zuChem has provided specification data for the Fourth Edition of the Food Chemicals Codex (FCC IV). The FCC IV specifications, along with results from analysis of five lots of mannitol, are presented in Table 1, below (these data are reproduced from Part D, Table 1, p. 000008 in the petition).

Table 1. FCC IV specifications for mannitol and compliance data for 5 lots of mannitol

		Lot Number				
Test	**FCC IV Specifications**	**M0803-001**	**M0803-004**	**M0803-005**	**M0803-006**	**M0803-007**
Identification	IR spectrum of sample exhibits maxima matching USP reference standard	Exhibits maxima	Exhibits maxima	Exhibits maxima	Exhibits maxima	Exhibits maxima
Assay	96-101.5%, dry weight	100.71%	100.42%	99.65%	99.79%	97.63%
Chloride	Not more than (nmt) 0.007%	Pass	Pass	Pass	Pass	Pass
Heavy Metals (as lead)	Nmt 5 mg/kg	Pass	Pass	Pass	Pass	Pass
Loss on drying	Nmt 0.3%	0.10%	0.14%	0.09%	0.16%	0.09%
Melting range	164-168°C	167°-168°C	167°-168°C	167°-168°C	167°-168°C	167°-168°C
Reducing sugars	Passes test	Pass	Pass	Pass	Pass	Pass
Specific rotation	Between +137° and +145°	+141.9°	+139.73°	+144.74°	+144.78°	+142.56°
Sulfate	Nmt 0.01%	Pass	Pass	Pass	Pass	Pass

ZuChem has also provided the following:

- Reproductions of the FCC IV mannitol monograph and incorporated FCC analytical methods (Appendix B, pp. 000024-50)
- Five certificates of analysis for five different lots confirming compliance with FCC IV specifications (Appendix C, pp. 000052-61)
- Report providing a discussion of specification tests performed, summary data, and raw data for assay (chromatograms) and identification (infrared spectra) tests (Appendix D, 000063-000101)

These data demonstrate conformance with the FCC IV specifications.

As stated previously, §180.25(b) requires compliance with the mannitol specifications listed in FCC III, not FCC IV. The specifications from the two editions of the FCC are compared in Table 2, below.

Table 2. Comparison of FCC III and FCC IV specifications for mannitol

Test	FCC III Specifications	FCC IV Specifications
Identification	Wet chemical test – Passes test	IR spectrum of sample exhibits maxima matching USP reference standard
Assay	96-101.1%, dry weight .	96-101.5%, dry weight
Arsenic	Not more than (nmt) 3 ppm	–
Chloride	Nmt 0.007%	Not more than (nmt) 0.007%
Heavy Metals (as lead)	Nmt 10 ppm	Nmt 5 mg/kg
Loss on drying	Nmt 0.3%	Nmt 0.3%
Melting range	165-168°C	164-168°C
Reducing sugars	Passes test	Passes test
Specific rotation	Between +23.3° and +24.3°	Between +137° and +145°
Sulfate	Nmt 0.01%	Nmt 0.01%

The specifications for mannitol in FCC III and FCC IV are fairly similar. The main differences are the following:

1. The identification test in FCC III is a wet chemical method, whereas FCC IV relies on infrared spectroscopy. This should not be a concern; the FCC IV identification test is equivalent to or better than that in FCC III.
2. The assay in FCC IV is slightly broader (96-101.5%, dry weight) compared to that in FCC III (96-101.1%, dry weight). This is not a concern. As can be seen in Table 1, mannitol produced from zuChem's process also complies with the FCC III assay specification.
3. FCC III contains a limit for arsenic, whereas FCC IV does not. ZuChem should verify that mannitol produced by their method complies with the FCC III specification for arsenic.
4. The FCC IV heavy metals (as lead) limit is more restrictive than that in FCC III. This is not a concern. Mannitol that is compliant with the FCC IV heavy metals (as lead) limit will also comply with the FCC III limit.
5. The melting range specification in FCC IV is slightly broader than that in FCC III. This is not a concern. As can be seen in Table 1, mannitol produced from zuChem's process also complies with the FCC III melting range specification.
6. The values for specific rotation are different. This is not a concern. Specific rotation values are dependent upon the solvent used to perform the test. The recommended solvent was changed between FCC III and FCC IV, resulting in different values for specific rotation. Mannitol that is compliant with the FCC IV specific rotation specification should also be compliant with the FCC III specification, when the differences in the solvent are taken into consideration.

ZuChem should provide evidence that mannitol manufactured using their process would comply with the FCC III arsenic limit of 3 ppm. ZuChem should also provide a general statement stating that mannitol produced by their method would meet the specifications of FCC III.

Use, Use Level, and Intended Technical Effect

ZuChem states that mannitol produced by their fermentation process is intended for use in accordance with the conditions stated in §180.25.

We have no questions.

ANALYTICAL METHODS

§180.25 does not specify an analytical method for demonstrating compliance with the regulated use levels. We expect that mannitol produced by fermentation using *Lactobacillus fermentum* could be detected in foods by the same analytical methods used to detect mannitol produced by one of the methods specified in §180.25(a).

We have no questions.

EXPOSURE ESTIMATE

Mannitol

Mannitol produced by the fermentation of *Lactobacillus fermentum* would be used in the same way as mannitol produced by one of the methods currently listed in §180.25. As a result, the use of mannitol produced by fermentation of *Lactobacillus fermentum* would be substitutional for mannitol produced by currently-regulated methods. Therefore, the exposure to mannitol would not increase.

Fermentation Organism

ZuChem states that no viable *Lactobacillus fermentum* organisms will remain in the final mannitol product produced by their method (Section E, p. 000009).

Based on this information, we agree that no viable *Lactobacillus fermentum* organisms would be present in the final mannitol product. As a result, there would be no exposure to the fermentation organism. However, we defer to the OFAS microbiologist for further comment on this issue.

Other sugar alcohols

ZuChem has not discussed the presence of sugar alcohols, other than mannitol, in the additive produced by fermentation of *Lactobacillus fermentum*. Chromatograms generated to show compliance with the FCC IV assay test (pp. 77-91) show, in addition to mannitol, the presence of sorbitol.

We request that zuChem comment on the presence of any sugar alcohols, other than mannitol, in the final product. Levels of these sugar alcohols should be provided, along with appropriate supporting data. ZuChem should also comment as to whether the identity and levels of the sugar alcohols present in mannitol produced by the petitioned method are comparable to those present in mannitol produced by the already-regulated

methods.

Proposed Regulation

The petitioner has proposed wording for the amendment of §180.25 to include mannitol produced by fermentation using *Lactobacillus fermentum* (p. 000006). This wording appears adequate.

Conclusion

ZuChem is petitioning to amend §180.25 to allow for the safe use of mannitol produced by the fermentation of sugars using *Lactobacillus fermentum.* We have requested data from zuChem regarding the compliance of mannitol produced by their process with FCC III specifications, as well as the concentrations of other sugar alcohols in the final mannitol product. When these requests have been addressed, we shall continue our review of the chemistry-related materials in the petition.

Daniel E. Folmer

Daniel E. Folmer, Ph.D.